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Corrigendum

Corrigendum to "Breakage and growth towards a stable aerobic granule size during the treatment of wastewater" [Water Res. 47 (2013) 5338–5349]

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Breakage and growth towards a stable aerobic granule size during the treatment of wastewater

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ABSTRACT

To better understand granule growth and breakage processes in aerobic granular sludge systems, the particle size of aerobic granules was tracked over 50 days of wastewater treatment within four sequencing batch reactors fed with abattoir wastewater. These experiments tested a novel hypothesis stating that granules equilibrate to a certain stable granule size (the critical size) which is determined by the influence of process conditions on the relative rates of granule growth and granule breakage or attrition. For granules that are larger than the critical size, granule breakage and attrition outweighs granule growth, and causes an overall reduction in granule size. For granules at the critical size, the overall growth and size reduction processes are balanced, and granule size is stable. For granules that are smaller than the critical size, granule growth outweighs granule breakage and attrition, and causes an overall increase in granule size. The experimental reactors were seeded with mature granules that were either small, medium, or large sized, these having respective median granule sizes of 425 μm, 900 μm and 1125 μm. An additional reactor was seeded with a mixture of the sized granules to represent the original source of the granular sludge. The experimental results were analysed together with results of a previous granule formation study that used mixed seeding of granules and floccular sludge. The analysis supported the critical size hypothesis and showed that granules in the reactors did equilibrate towards a common critical size of around 600–800 μm. Accordingly, it is expected that aerobic granular reactors at steady-state operation are likely to have granule size distributions around a characteristic critical size. Additionally, the results support that maintaining a quantity of granules above a particular size is important for granule formation during start-up and for process stability of aerobic granule systems. Hence, biomass washout needs to be carefully managed to optimize granule formation during the reactor start-up.

1. Introduction

Aerobic granular sludge systems are an emerging technology where aerobic granules are utilised as opposed to floccular sludge for activated sludge wastewater treatment (de Bruin et al., 2004; Liu et al., 2005a; de Kreuk et al., 2005). A major advantage of the relatively large and dense granules is that much faster sludge settling rates can be achieved in

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comparison to conventional plants, thus greatly reducing plant footprint and capital cost (de Bruin et al., 2004; Liu and Tay, 2004). Compared to floccular sludge, granular sludge achieves high biomass retention, better handles shock loads and higher loading rates (Liu and Tay, 2004) and shows improved sludge dewaterability (de Kreuk et al., 2005). Paramount to the performance of aerobic granular sludge systems is a stable, dense and reasonably large (0.5–1.5 mm) granule size (Beun and Hendrik, 1999). In reactors, average granule sizes between 0.2 and 5 mm are reported and this size is governed by a combination of growth increasing the granule size, and attrition/breakage processes decreasing its size (Liu and Tay, 2004).

The mechanisms by which granules form and grow have been under examination (Ahn et al., 2009; Barr et al., 2010; Verawaty et al., 2012; Wan et al., 2011; Ni et al., 2010). Aerobic granules are suspended biofilms and models describing their size include processes of attachment and detachment, cell growth, EPS production, cell death and lysis, and predation by higher organisms (Beun et al., 2002; Picioreanu, 1999). Granule growth is suggested to occur by single colony outgrowth or by the aggregation of multiple smaller colonies (Barr et al., 2010; Verawaty et al., 2012) and by layering of cells attaching to a surface (Hulshoff Pol et al., 2006). Seeding of aerobic granular sludge reactors with specific microbial strains (Ivanov et al., 2008) or with crushed aerobic granules and floccular sludge can accelerate granule formation (Verawaty et al., 2012; Pijuan et al., 2011). These findings suggest that seed biomass, including granule fragments, can enhance granule formation.

A feature of biological granules is that they consist of mixed microbial populations imbedded in a three-dimensional glue of extracellular polymeric substances (EPS). The EPS plays a crucial role in determining granule stability (Liu and Tay, 2004) and is described as a hydrogel-like substance (Seviour et al., 2009). Biological activity occurs throughout a granule (not just on the granule surface) and is expected to impact the long-term growth (increase in size), strength and tendency to break (decrease in size). The three-dimensional structure of granules would cause stratification of microorganism types and metabolism, due to a combination of diffusive mass transfer into the granule and biological activity consuming and producing metabolites (Tay et al., 2003). Consequently, substrate limitations within the centres of large granules may alter biological activity and lead to a weakened granule structure (Liu et al., 2005a; Toh et al., 2003). Additionally, larger particles in mixed suspensions will have greater impact energy associated with collisions. As a consequence of these events, the rate at which aerobic granules wear would increase with their size.

Granule structure and size are critical for the performance of an aerobic granular sludge system. However, presently there is limited understanding of the dynamics of particle size for aerobic granule systems. Individual granules in an aerobic reactor are part of a particle population that will comprise variation in size, growth and strength characteristics. To date, there has been limited consideration of the entire aerobic granule population in the study of growth and size reduction processes.

The present study investigates the influence of granule size on breakage/fragmentation propensity and in-turn the influence of granule breakage on the resulting long-term granule size distribution. Here it is hypothesized that within an aerobic granular sludge system, a certain "critical" granule size will be achieved at steady state under certain operational conditions (wastewater characteristics, aeration, reactor geometry, mixing, and solids concentration). Due to the consequences of particle collision energies and structural weaknesses, granules larger than the critical size are expected to break/attrite until reduced to the critical size or smaller. This rate of breakage/attrition of the large granules is exceeding the rate at which they grow. Conversely, the growth of small granules exceeds the rate at which their size is being reduced by breakage or attrition until they reach the critical size. To test this Critical Size hypothesis, the present study followed the change in the size of granules of various starting sizes over 50 days in separate sequencing batch reactors (SBR). The experimental design considers granule populations in their entirety.

2. Materials and methods

2.1. Preparation of aerobic granule seed sludge

Aerobic granules used as seed sludge in the experiments were sourced from a 5L lab-scale SBR called the parent reactor or SBRp. The SBRp was operated on an 8-h cycle consisting of stages of static fill (2 min), anaerobic (78 min), aerobic (305 min), anoxic (90 min), settling (2 min) and decanting (3 min), as previously described (Yilmaz et al., 2008). This parent reactor treated abattoir wastewater for biological nutrient removal (BNR). The abattoir wastewater feed contained soluble chemical oxygen demand (COD) at 862–1137 mg/L, volatile fatty acids (VFA) at 650–800 mg/L, ammonia nitrogen (NH_4^+–N) at 200–254 mg/L, and orthophosphate phosphorus (PO_4^3–P) at 31–40 mg/L. The wastewater volumetric exchange ratio (VER) was 50%, resulting in a hydraulic residence time (HRT) of 16 h. Mixed liquor suspended solids (MLSS) and volatile MLSS (MLVSS) were 20.2 g/L and 18.19 g/L respectively. The sludge retention time (SRT) was kept between 15 and 20 days by wasting sludge at the end of the aerated phase.

The entire sludge content of SBRp was fractionated into five different size fractions using standard sieves with apertures of 160 μm, 350 μm, 500 μm, 700 μm, 850 μm, 1000 μm and 1180 μm. From these, three fractions were chosen, large, medium and small, to be used as the seed sludge for the operation of the experimental SBRs (Table 1).

Table 1 – Size fractionation of mature aerobic granules from SBRp.

Sieving processes	Seeds name	Particle size distributions (μm)			Biomass obtained (g VSS)
		d (0.1)	*d* (0.5)	*d* (0.9)	
>1800	Large	700	1125	1550	48.19
<700, >500	Medium	606	900	1346	8.5
<500, >160	Small	164	425	763	2.3

2.2. Experimental reactor operation

Four experimental SBRs were operated to test the Critical Size hypothesis. Three of the lab-scale SBRs were seeded individually with the different size granule seeds, SBRL with large granules, SBRM with the medium granules, and SBRSmall with the small granules. As a control reactor operation, a seed sludge was prepared that resembled the granule size distribution of the original parent reactor, called Mix. This seed comprised a mixture of the various size fractions being 5% small, 45% medium, and 50% of the large size granules (on a dry weight basis), with the composition of Mix being based on the percentage of each of these separate fractions in the total biomass of SBRp (Table 1).

The working volume for each experimental SBR was 1.5 L and granules were added so the initial MLSS for each was 1.4 g/L. During each cycle of the SBR operations these were fed 100 mL of the same VFA and abattoir wastewater mix provided to SBRp (Pijuan et al., 2011). Consequently, the F/M ratio of the experimental SBRs was 0.14 g COD/g MLVSS/day, which was similar to that of SBRp at 0.10 g COD/g MLVSS/day. The SBR cycle length was extended to 10 h and consisted of: feed (1 min), anaerobic (189 min), aerobic (358 min), anoxic (45min), settling time (2 min for SBRL and SBRM and 5 min for SBRSmall and SBRMix to avoid biomass washout), idle (3 min for SBRL and SBRM, 0 min for SBRSmall and SBRMix) and decanting (2 min). Other reactor operating conditions were kept identical to that of SBRp. The experimental SBRs were operated for 50 days and 30 mL mixed liquor samples were collected every 2 or 3 days for the size distribution analyses described below. To minimise the removal of biomass by sampling over time, the majority of the 30 mL mixed liquor samples were returned back into the reactors after the size measurement. To track the biomass content and nutrient removal performance, MLSS and MLVSS were measured every 2 or 3 days, followed by a cycle study conducted every 2 weeks. To track the microbial composition of the sludge, samples for FISH analysis were fixed during cycle studies but only samples of interest were analysed.

As noted in the results section below, SBRSmall showed poor biological treatment performance, and so the size analysis for this particular reactor was not considered valid for the testing of the Critical Size hypothesis. Consequently, the dataset of the present experiments was augmented with the data from an experiment previously reported by the authors (Pijuan et al., 2011). However, the present study reports additional and unique analysis of the previous dataset that has not been published before. The particular experiment of Pijuan et al. (2011) for which data was analysed, involved seeding of an SBR (from now on called SBRSmall_Pijuan) with a mixture of 30% of crushed granules and 70% floccular sludge. The crushed granules were prepared by pressing intact granules through a certified sieve with a pore size of 180 μm. The seed granules of SBRSmall_Pijuan were of small size (median size of 235 μm) and these granules were mixed with a floccular sludge such that the median particle size was 75 μm. SBRSmall_Pijuan was seeded with granules of similar size to SBRSmall (median size of 425 μm) in comparison to the much larger seed sizes used for SBRM and SBRL. The reactor SBRSmall_Pijuan was operated under very similar conditions to the experimental reactors of the present study. Both SBRSmall_Pijuan and SBRSmall were fed with abattoir wastewater. SBRSmall_Pijuan was operated at a 2 L working volume (as opposed to the 1.5 L working volume of SBRSmall) and a starting MLVSS of 3 g/L (as opposed to 1.4 g/L for SBRSmall). Pijuan et al. also increased wastewater loading per cycle gradually from 0.25 to 0.5 L at the beginning of the reactor operation to 1 L towards the end of the experimental run (Pijuan et al., 2011). Given the similarities of the reactor experiments (SBRSmall and SBRSmall_Pijuan) and that SBRSmall_Pijuan maintained biological nutrient removal performance during its operation, the data from SBRSmall_Pijuan was included in this study for testing the critical size hypothesis.

2.3. Analytical techniques

The size of granules in the mixed liquor samples was measured by laser diffraction (Malvern Mastersizer, 2000 series, version 5.60, Malvern Instruments Ltd, Malvern, UK) with tap water as the suspension medium and standard optical parameters. Granule morphology was visualized using an Olympus SZH10 microscope (Olympus Optical CO, LTD).

Ammonia (NH_4^+–N), nitrate (NO_3–N), nitrite (NO_2–N) nitrogen and phosphate phosporus (PO_4^3–P) concentrations were analysed for filtered samples of reactor contents filtered at (0.22 μm) using a Lachat QuikChem 8000 Flow Injection Analyser (Lachat Instrument Milwaukee. Wisconsin). Volatile fatty acids (VFAs) in the same filtrate were measured by gas chromatography (Perkin–Elmer) with column DB-FFAP 15 m; 0.53 mm; 1.0 mm (length; ID; film) at 140 °C. Total and soluble COD, MLSS and MLVSS concentrations were determined according to the standard methods (APHA, 2005).

To perform microbial community analysis, fluorescence in situ hybridisation (FISH) was conducted on fixed samples of granular sludge as previously described (Amann, 1995). Oligonucleotide probes used were: Cy5-labelled EUBmix for the detection of all bacteria; Cy3-labelled PAOmix probes for detection of *Candidatus* Accumulibacter phosphatis, (a widely reported polyphosphate accumulating organism, PAO), comprising equal amounts of probes PAO462, PAO651 and PAO846 (Crocetti et al., 2000); and Cy3-labelled GAOmix probes for the detection of *Candidatus* Competibacter phosphatis (a well-known glycogen accumulating organism, GAO), comprising equal amounts of GAOQ989 (Crocetti et al., 2002) and GB_G2 (Kong et al., 2002). FISH preparations were visualized with a Zeiss LSM 510 Meta confocal laser scanning microscope (CLSM) using a Plan-Aphochromat 63× oil (NA 1.4) objective. Cy3 and Cy5 were excited with a HeNe laser (543 nm) and a red diode laser (637 nm) and collected with 550–625 nm BP and 660 nm LP emission filters respectively. Twenty images were taken from each sample for quantification of PAO and GAO, these were calculated as a percentage of the total bacterial population using the image analysis software *daime* version 1.2 (Daims et al., 2006).

2.4. Analysis of size distribution data

The sizing technique employed by the Malvern Mastersizer instrument described above provides particle size

distributions in terms of a volume fraction in a size interval with respect to the volume equivalent size. The volume equivalent size is the diameter of a spherical particle with the same volume as the particle being sized and the volume fraction in a size interval expresses the fraction of the total volume of granules with sizes falling within a specific particle size interval. To maximise clarity when presenting data for the entire particle size range covered by the Malvern instrument, the instrument typically plots measured volume fractions against particle size on a logarithmic scale, and outputs tabulated data accordingly. To better visualize the data in the present study, the size data was converted to volume frequencies for presentation on a linear size access. Consequently, the size data are then presented as volume frequency distributions (the relevant statistical density function). These were obtained by dividing the size fraction obtained from the Malvern instrument for each size interval by the width of that size interval (upper size of the size interval minus the lower size of the size interval). The granule size distributions were then presented below in their entirety to fully benefit from transparent experimental data and were also summarised in terms of three main statistical size parameters:

1. the median size or D(50) of the size distribution – 50% of the total particle volume consists of particles larger than the D(50) and 50% of the total particle volume consists of particles smaller than the D(50);
2. other percentiles for the size distribution, in particular the D(10) and D(90) which are similar to the D(50), in that 10% and 90% of the total particle volume have a smaller particle size than the D(10) and D(90), respectively; and
3. the mode of the size distribution, which is the particle size with the largest corresponding volume frequency.

3. Results

3.1. *Biological treatment performance of the experimental reactors*

Aerobic granules used as the seed sludge in the experimental reactors were sourced from a 5L lab-scale SBR (the SBRp), which treated abattoir wastewater for BNR. At the time of harvesting the seed sludge, the parent reactor was achieving stable removal of soluble COD, soluble N and soluble P from abattoir wastewater with efficiencies of 85%, 95% and 85% respectively. The granules had a stable size distribution with a median size of 800 μm.

The experimental reactors seeded with the large size fraction granules (SBRL), the medium size fraction granules (SBRM) and the size fraction mix (SBRMix) achieved biological nutrient removal throughout the operating period. For these SBRs the respective average levels of N removal were 79%, 87% and 86% and the respective levels of P removal were 49%, 29% and 30% (Fig. 1). The MLVSS in SBRL and SBRM were reasonably stable and averaged at 2.06 g/L and 2.3 g/L, respectively. Although lower, the MLVSS in SBRSmall reached 1.6 g/L on day 32. However, for reasons discussed below, SBRSmall had comparatively poor BNR performance (Fig. 1). Additionally, the proportions of PAOs and GAOs, as quantified by FISH, were much lower in SBRSmall than in the other SBRs (Table S1, Supplementary Information) indicating a substantial microbial community change had occurred.

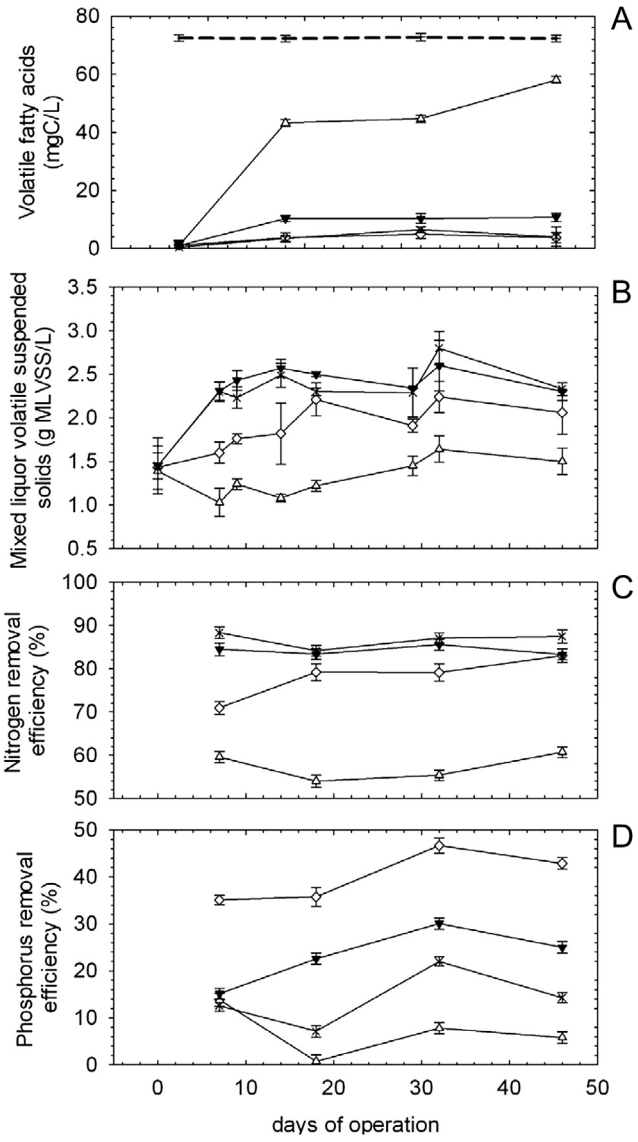

Fig. 1 – Biological treatment performance for the operation of the experimental sequencing batch reactors (SBRs), including; (A) SBR levels of volatile fatty acids at the end of the anaerobic stage, (B) mixed liquor volatile suspended solids, (C) nitrogen removal efficiency, and (D) phosphorus removal efficiency. Data are shown for reactors seeded with large granules (SBRL, open diamonds), medium sized granules (SBRM, crosses), small granules (SBRSmall, upright open triangles) and mixed sized granules (SBRMix, closed triangles). The mixed liquor VFA concentration directly after feed addition was 75 mg/L, shown as a dashed line (A). Error bars indicate standard error on individual measurements.

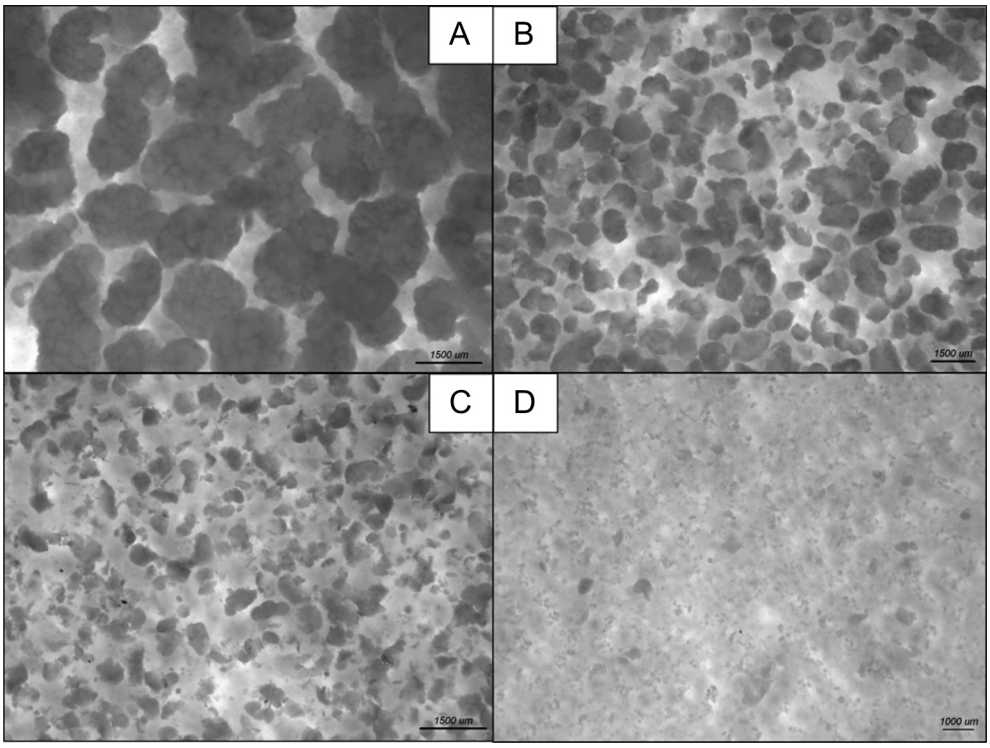

Fig. 2 – Light microscope images of granules used to seed the experimental reactors: (A) SBRL, (B) SBRM (C) SBRSmall, and (D) crushed granules before mixing with floccules as was used to seed SBRSmall_Pijuan. The scale-bars on all images indicate 1.5 mm., excepting for D where the scale bar is 1000 μm

3.2. *Changes in granule size distributions over the treatment period*

The experimental SBRs were started with the different sized granule fractions (Table 1, Fig. 2), with SBRMix and SBRM having large particle size distributions most similar to SBRp (Fig. 3A). The SBRSmall_Pijuan, the granule/floccule mix, had the smallest particle size at the start of operation (Fig. 3B, Table 2). The size distribution of SBRp had a median granule size of 800 μm. The small, medium, and large size fractions had median granule size of 425 μm, 900 μm, and 1125 μm, respectively (Table 2).

Particle size distributions were measured over time for each of the experimental SBRs and these were compared to the size distribution of SBRp (Fig. 4). Summary statistics are given as timetrend plots in Fig. 5 with Fig. 5E including an expanded dataset for the experiment SBRSmall_Pijuan. Granules in SBRL were seen to decrease in size over time (Fig. 5A) and the granule size distributions were bimodal (Fig. 4A). The emerging separate peak for the smaller particles was thought to be granule fragments, and the peak with larger diameter was the remaining seed granules (see the modal peak sizes in Table 2). The overall trend in the particle size distribution for SBRL was a decrease in the amount of larger particles due to fragmentation and an increase in the amount of smaller particles as fragment offspring. However, as shown by a comparison of the size distributions at day 10 and 20 (Fig. 4A), some of the smaller fragments had washed out, causing an incremental decrease in the smaller particles by day 20. This is also reflected by an increase in the percentile values between day 10 and 20 (See Fig. 5A). Near the completion of the treatment period (days 45–50), the peak in size distribution corresponding to the large particles in SBRL closely resembled that of the SBRp (Fig. 4A).

For granules of SBRM, the reactor seeded with the medium sized fraction (with median size expected to be near the critical size), the size distribution was seen to spread during the operation time (Figs. 4B and 5B). The median size of the granule size distribution of SBRM decreased slightly over time due to the formation of smaller particles (compare seed with 50 day sludge in Table 2). The final granule size distribution for SBRM appeared to be very similar to that of the original sludge of SBRp (Fig. 4B).

Granules of SBRSmall (the reactor seeded with the small size fraction) seemed to disappear from the particle size distribution over time (Figs. 4C and 5C), likely being washed out from the reactor. The biomass of SBRSmall was reduced from 1.4 g/L at the start of operation, to around 1.1 g/L by days 10–20 (Fig. 1B), and this resulted in VFA being present at the end of the anaerobic stage (Fig. 1A). A new population of smaller particles formed by day 20 (Fig. 4C) with a modal peak size of around 80 μm. At the end of the treatment period, the overall size distribution of SBRSmall was clearly much smaller than that of the parent reactor SBRp (See day 50, Fig. 4C, Fig. 5C). These observations were thought to be an effect of the poor biological performance of SBRSmall, as is discussed below.

Results from an additional experiment of Pijuan et al. (2011) were further analysed to augment the failed operation

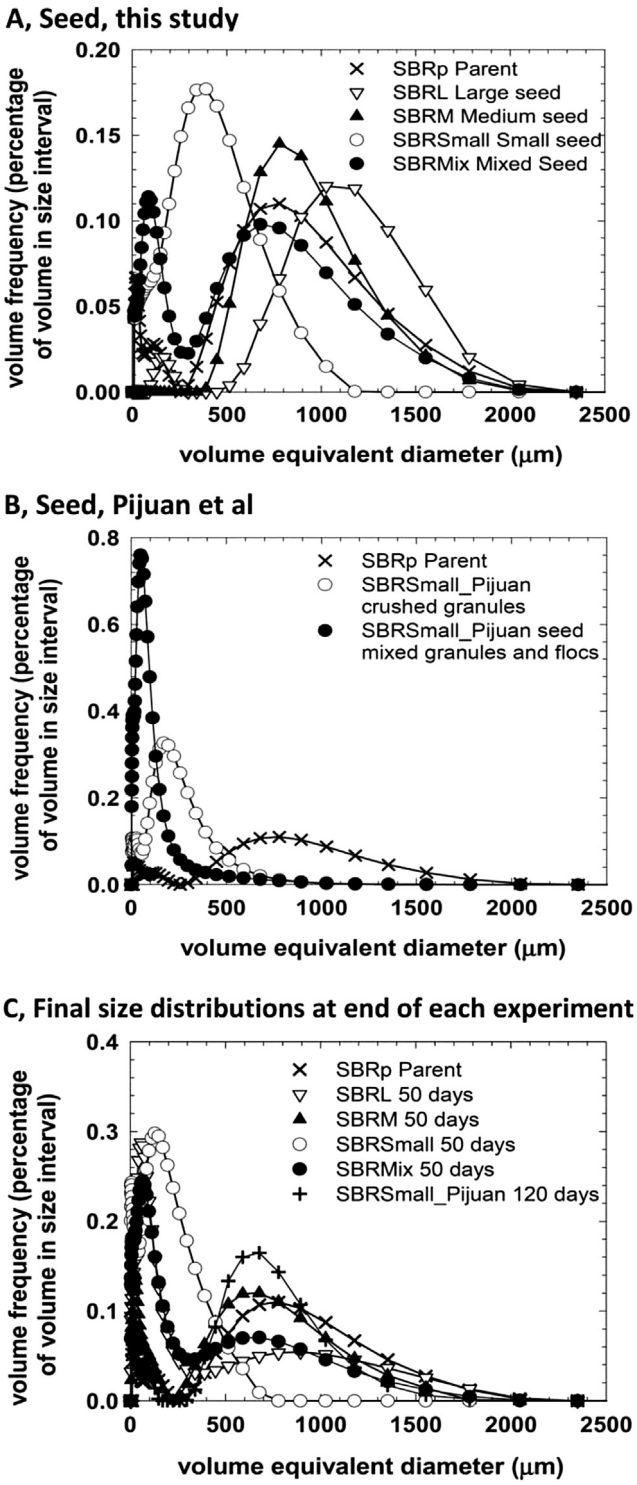

Fig. 3 – Particle size distributions of the parent reactor SBRp plotted together with that of (A) granules used to seed the experimental reactors SBRL, SBRM, SBRSmall, and SBRMix, (B) crushed granules before and after mixing with floccules used to seed SBRSmall_Pijuan, and (C) size distributions measured at the end of each experimental SBR run. Note the change of scale on the volume frequency axes.

of SBRSmall. That experiment (SBRSmall_Pijuan) used a mixture of 30% crushed granules and 70% floccular sludge as seed material. The size distributions measured for SBRSmall_Pijuan show a clear increase in particle size, indicating growth of the sludge (Fig. 4D). Similar to SBRSmall, biomass loss did occur during the initial stages of SBRSmall_Pijuan operation (Pijuan et al., 2011). However, on this occasion, possibly due to the higher initial biomass of 3 g/L (MLVSS), granules remained and developed in SBRSmall_Pijuan. At around day 75 the size distribution became bimodal (Fig. 4D), which was thought to represent the original small particle sludge (peak with smaller size) and the development of a larger granular sludge (peak with larger size). From day 75 to day 120, the amount of the larger granular sludge increased as indicated by an increase in the height of the peak for the larger particles, and a decrease in the height of the peak for the smaller particles (Fig. 4D). This trend is clearly seen from a rapid increase in the median size particles from day 70 to day 80 (Fig. 5E), and Pijuan et al. (2011) identified day 80 as the "granulation time". The final size distribution of SBRSmall_Pijuan closely resembled that of SBRp.

To further examine and verify the experimental approach, a reactor (SBRMix) was seeded with a mixture of small, medium and large granules to resemble the overall size distribution of the original sludge in the parent reactor SBRp. Similar to SBRM, granules of SBRMix were observed to undergo size spreading over time (Figs. 4E and 5D). Similar to SBRL, smaller particles formed in SBRMix over time as shown by the development of a bimodal size distribution. The bimodal distribution had respective modal peak sizes of 680 μm for the larger granules and 60 μm for the smaller particles. The smaller particles were thought to have originated from breakage of some of the larger granules. Near the end of the test (day 50) the portion of the size distribution corresponding to the larger granules in SBRMix, closely resembled the overall size distribution of the parent SBRp (Figs. 4E and 3C), suggesting that the larger particles in SBRMix had a comparable steady-state size to the granules of SBRp.

With the exception of SBRSmall, size distributions at the end of each experimental reactor run were bimodal (Fig. 3C). The mode size of the peak corresponding to larger particles in the bimodal size distributions was similar for all the reactors at the end of operation and similar to that in SBRp (Fig. 3C, Table 2).

4. Discussion

4.1. *Granule growth and breakage towards a steady state size*

Based on the present study and results from previous research, a conceptual model was developed for how aerobic granules grow and break/attrite towards a stable/steady-state size (Fig. 6). Here the model is discussed to provide insight and hypotheses for granule size dynamics within aerobic activated sludge systems.

The initial granule formation process is said to occur by the aggregation of smaller biological particles to form dense clumps (Ahn et al., 2009; Barr et al., 2010; Verawaty et al., 2012; Wan et al., 2011). Further growth then occurs by layered

Table 2 – Summary size characteristics for the parent and experimental sequencing batch reactors.

Reactor	[a]Median [b]size of seed (μm)	Median [b]size at the end of experiment (μm)	[c]Modal size of smaller particles when bimodal peaks at the end of experiment (μm)	[c]Modal size of larger particles when bimodal peaks at the end of experiment (μm)
SBRp	–	800	–	–
SBRL	1125	680	60	680
SBRM	900	800	25	680
SBRSmall	425	230	–	–
SBRSmall_Pijuan, Granules/floc mix	75[d]	720	85	650
SBRMix	–	620	60	680

a Typical uncertainty on each size listed in the table is ±10 μm.
b The median sizes reported here are for volume-based particle size distributions, i.e. 50% of the particle volume lies on either side of the median size.
c The peak modal sizes were examined in cases where the overall size distribution was bimodal in shape and the peak size corresponds to a peak volume frequency for the respective modes of the bimodal size distribution. The modal size for the larger particles is only given to show the two distinct peaks with respective modal sizes.
d After combining crushed granules (30%) with floccular sludge (70%) – Seed for SBRSmall_Pijuan.

outgrowth of micro-colonies, as well as by aggregation where collisions between granules and cohesion produce a lasting aggregate of larger size (Barr et al., 2010; Verawaty et al., 2012). Prior investigations have accelerated granule formation by seeding reactors with crushed granules and floccular sludge (Pijuan et al., 2011), and aggregation is detected for initial granule expansion (Verawaty et al., 2012). During the early stages of granule formation, this stepwise increase in size may provide the mechanism for maintaining biomass within reactors during selection for aerobic granules.

It is expected that aerobic granules will undergo wear by erosion or abrasion in dense settled slurries or as a result of collisions with reactor components or other granules in suspension. Breakage/fragmentation and wear/attrition all cause a decrease in granule size and as described in the introduction, larger granules will be more prone to breakage than smaller granules. This is partly due to substrate limitations leading to a weakened inner core of the granule (Liu et al., 2005a; Toh et al., 2003). The main difference with respect to influence on granule size dynamics is that breakage/fragmentation causes a step decrease in granule size (disappearing of large granules, appearing of a large number of small granules), whereas the size reduction for wear/attrition would be more incremental (Litster and Ennis, 2004).

Both granule growth and granule size reduction by fragmentation/erosion influenced the evolving granule size distribution in SBRM (Fig. 4B). In this reactor, spreading (widening) of the size distribution was observed, with the median size remaining largely unchanged over the experimental period. This suggests that granule growth (distribution spreading towards larger size) and granule breakage/erosion (distribution spreading towards smaller size) were occurring simultaneously and were balanced. The size reduction effects of granule fragmentation/wear were clearly observed with SBRL from the movement of the size distribution in the direction of smaller size (Figs. 4A and 5A) and also by the appearance of smaller particles over time. The smaller particles were likely fragmentation products of the larger granules. A combination of the effects described for SBRL and SBRM were observed with SBRMix where the size distribution for larger granules spread over time and distinct smaller particles were generated over time (Fig. 4E). These observations supported the conceptual model of growth and size reduction processes outlined in Fig. 6.

It is proposed that the fragmentation of larger granules will act as a viable seed material by subsequent re-growth of fragments (Lemaire et al., 2008). Crushed granular sludge has previously been shown to act as a good seed material for the rapid start-up of granular sludge systems (Pijuan et al., 2011). However, the findings of the present study with SBRSmall (where smaller granules/granule fragments were added as seed) highlight an important consideration with respect to seed sludges. The biological performance of SBRSmall was poor, thought to be due to the seed sludge having poor activity or being too small/ill-shaped/not sufficiently dense to be retained in the reactor. The initial loss of viable biomass from SBRSmall resulted in most of the VFAs not being utilised in the anaerobic phase (Fig. 1A). These events likely caused the poor nutrient removal performance in SBRSmall (Fig. 1). The subsequent utilisation of VFAs in the aerobic phase would cause major changes to the ecology of the sludge (as was detected by FISH) and cause changes to the granule morphology and structure, as observed previously (McSwain et al., 2004). These morphology and structure changes would likely contribute to the loss of those granules from SBRSmall. These findings further highlight the need to consider the composition of the seed material in terms of particle size, biological activity and organic loading in the application of the technology.

The small-granule and floccular sludge mix used in the experiment of Pijuan et al. (2011) was a viable seed, resulting in successful granulation and reactor performance for SBRSmall_Pijuan. Interestingly, the appearance of the bimodal distribution was quite pronounced at day 75 (Fig. 4D). At this time there was a sudden disappearance of smaller particles and a sudden appearance of larger granules. This suggests that the granular growth is occurring here at least by aggregation, which has been reported previously (Barr et al., 2010; Verawaty et al., 2012).

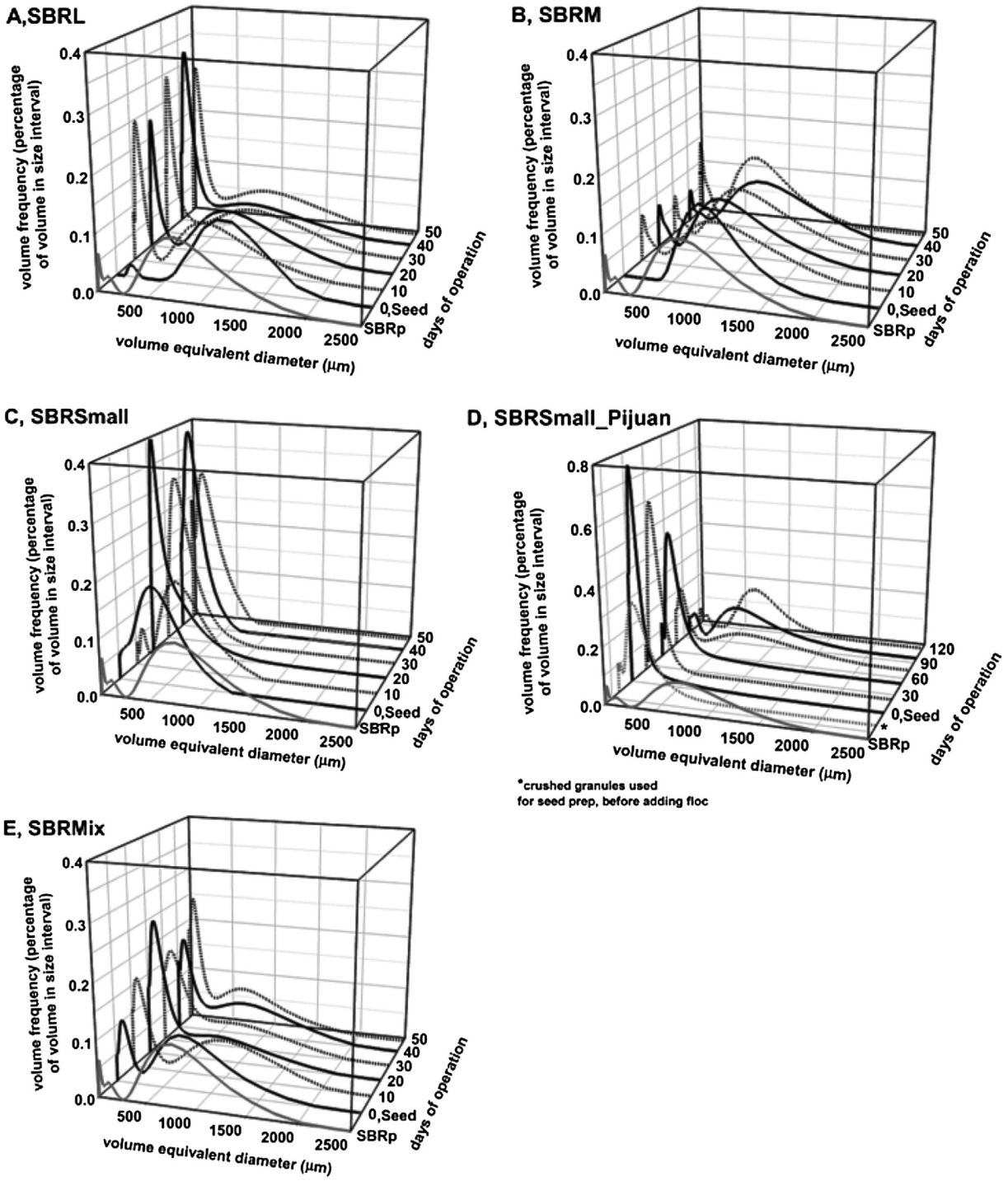

Fig. 4 – Granule size distributions plotted against operational time for the experimental SBR reactors which were seeded with (A) the large sized seed fraction (SBRL), (B) the medium sized seed fraction (SBRM), (C) the small sized seed fraction (SBRSmall), (D) the mix of crushed granule and floccular sludge (SBRSmall_Pijuan), and (E) the mixed sized granule seed fraction (SBRMix). The granule size distribution of the parent reactor SBRp is also shown as a grey line in each figure for comparison.

4.2. *The critical size hypothesis*

In the present study it was hypothesised that a certain critical granule size exists for particular process operating conditions. Relevant operating conditions may include wastewater characteristics, settling speed, shear forces, mode of feed and aeration cycles (Liu et al., 2005a; Ahn et al., 2009; McSwain et al., 2004). For reasons given above, granules larger than the critical size would be more prone to undergo breakage or attrition, such that size reduction outweighs growth, and

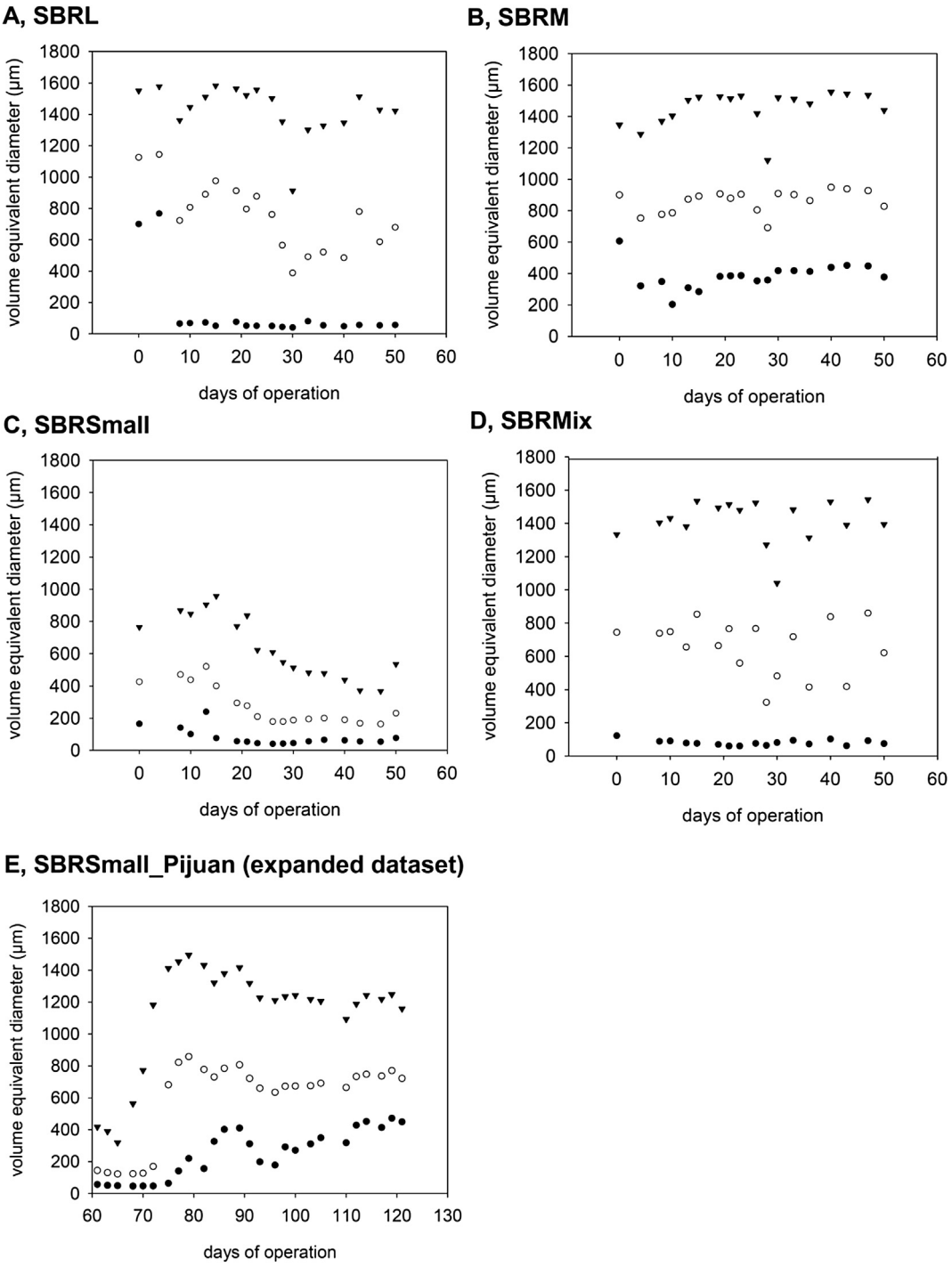

Fig. 5 – Timetrend plots of percentiles for measured granule size distributions, giving the median size D(50) (open circles), 10th percentile D(10) (closed circles), and 90th percentile D(90) (upside down triangles) of the experimental SBRs and the expanded dataset of SBRSmall_Pijuan.

these granules decrease down to the critical size. Conversely, for granules smaller than the critical size it is expected that growth outweighs size reduction and the net result is an increase in granule size up to the critical size. The overall effect is a steady-state granule size distribution consisting of a spread of sizes around the dominant critical size.

Observations in SBRL, SBRM and SBRMix, and SBRSmall_Pijuan supported the above hypothesis as the granules in these SBRs all equilibrated towards a final critical size of between 600 and 800 μm. For reasons given above, the results for SBRSmall were not considered valid for the testing of the Critical Size hypothesis, and were thus disregarded. The basis of the test was that the original parent reactor SBRp was thought to have a steady-state size distribution spread around the critical size, which would be similar to the steady-state critical size of the experimental SBRs. As shown in Fig. 3A,

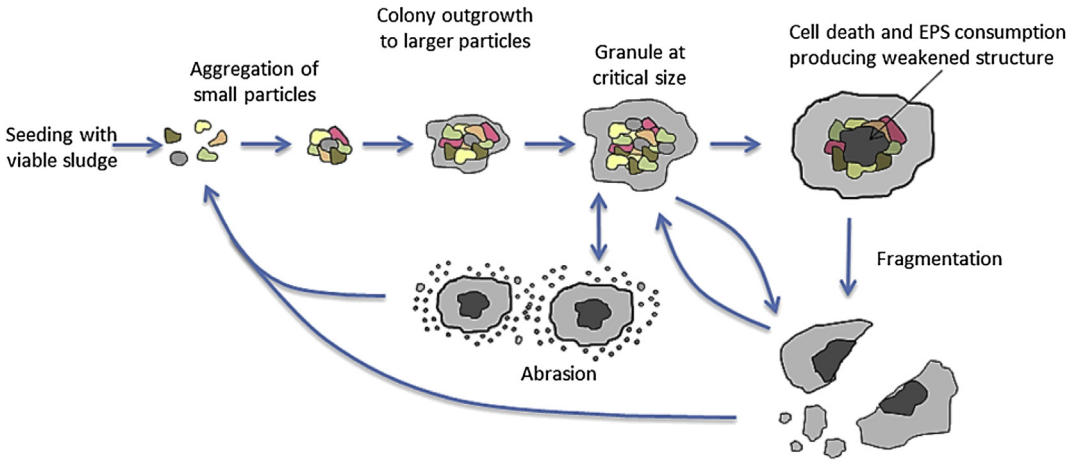

Fig. 6 – A depiction of the conceptual model for granule formation and breakage/attrition.

seed granules for SBRL were mostly larger than granules from SBRp, that is, larger than the critical size for similar operating conditions to the experimental reactors. Also, at day 10, granules in SBRMix were visibly larger than granules in SBRp (Fig. 4E), likely due to granule growth. As expected from the hypothesis, size reduction then caused a migration of the granule size distribution for SBRL and SBRMix (Fig. 4A and E) towards the original position of the size distribution of SBRp (the critical size). On days 45–50 (final, steady state size), the portion of the bimodal final size distribution of SBRL corresponding to large granules, closely resembled the size distribution of SBRp (Fig. 4A). The reactors SBRL and SBRMix still contained a large quantity of smaller particles at the end of the treatment period (Fig. 4A and E), so the treatment period was thought to be too short to allow the granule size distribution of SBRMix to completely equilibrate. Regardless, the modal size distribution of the larger particles in SBRMix (Fig. 4E) closely resembled the size distribution of SBRp (Fig. 3C), again supporting the critical size hypothesis. Seed granules for SBRM had a median size comparable to that of SBRp (Fig. 3A), which was the critical size for these SBRs operating under similar conditions. Over time, the size distribution of SBRM spread (Figs. 4B and 5B), with the median size reducing only slightly when compared with the median size change noted for SBRL (Table 2). This spreading of the size distribution was thought to represent statistical differences in the rates at which individual granules in the population grew or reduced in size. Overall, the results suggest that the critical size for the experimental reactors was similar, or only slightly smaller than that of SBRp. The slight difference in the critical size possibly resulted from small differences in the SBR operational conditions. In comparison, SBRp had a larger working volume, slightly lower F/M ratio, and a shorter SBR cycle (an 8 h cycle as opposed to a 10 h cycle).

4.3. *Proposed practical implications*

Particular granule sizes are suggested for the optimal operation of aerobic granular systems. Granules of diameter range between 1.0 and 3.0 mm (Wang et al., 2007) and 2.0–3.0 mm (Toh et al., 2003) have been recommended. Although, it is thought that within aerobic granules larger than 700 μm, much of the biomass will be metabolically restricted due to limitations of mass transfer (Liu et al., 2005b). Attempts for control of granule size have been achieved in these systems by selectively wasting particular size fractions (Li et al., 2006). Nonetheless, achieving a particular size of mature granules in a stable system will depend on various physical and operational conditions of the reactor, as well as the wastewater composition.

Maintaining granule size near the critical size is likely important for stable operation of a full-scale aerobic granular sludge WWTP. Performance failure was observed here when the starting granule size was well below the critical size in SBRSmall. The selection for granules by use of a short settling phase resulted in washout of biomass and SBRSmall became floccular. This is relevant to the start-up period of an aerobic granule reactor when granule formation occurs. It is observed that granular reactors are successfully started with seed granules of various starting sizes. However, results from the present study suggest that if the granule size is below the critical size, then sufficient biomass levels need to be maintained to avoid granule loss and reversion to a floccular sludge. It is suggested that this was achieved in SBRSmall_Pijuan by seeding the reactor with a relatively higher MLVSS in comparison to SBRSmall. Consequently, during operation of reactors that are selecting for granules from a floccular system, control measures such decreasing the sludge settling period need to be carefully manipulated to maintain sufficient biomass levels.

5. Conclusions

This experimental study provides new insight into granule size dynamics that occur during the operation of aerobic granular sludge reactors. From the detected changes in granule size a conceptual model was proposed describing how granules grow up to a certain critical size, and how granules that have managed to grow larger than the critical size tend to

break/attrite and this way reduce in size down to the critical size. The consequence is a steady-state distribution of granule sizes spread around the critical size corresponding to the particular operating conditions of the aerobic granular sludge reactor (wastewater characteristics, aeration, reactor geometry, mixing, and solids concentration) which determine the respective rates of growth and size reduction processes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2013.06.012.

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